51000 Nesults 510091911,132

·		<u> </u>	·	PN 16
L Number	Hits	Search Text	DB	Time stamp
1	235	(((multiple or several or different or	USPAT;	2003/08/27
		many or independent) adj (transformant or	US-PGPUB;	07:49
		transformation)) same (resistance or	EPO; JPO;	
		selection)) and ((high or increased or	DERWENT;	
		efficient) adj3 (express or expression))	IBM_TDB	
2	349	1 ' ' '	USPAT;	2003/08/27
		many or independent) adj3 (transformant	US-PGPUB;	07:52
		or transformation)) same (resistance or	EPO; JPO;	
		selection)) and ((high or increased or	DERWENT;	
		efficient) adj3 (express or expression))	IBM_TDB	0000/00/0=
3	42	1 ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' '	USPAT;	2003/08/27
		or expression) and (((multiple or	US-PGPUB;	07:53
		several or different or many or independent) adi3 (transformant or	EPO; JPO;	
		<pre>independent) adj3 (transformant or transformation)) same (resistance or</pre>	DERWENT; IBM TDB	
		selection)) and ((high or increased or	TDII_IDD	
[i		efficient) adj3 (express or expression)))		
		and yeast		
4	86	1 "	USPAT;	2003/08/27
-		many or independent) adj3 (transformant	US-PGPUB;	07:52
		or transformation)) same (resistance or	EPO; JPO;	0.102
1		selection)) and ((high or increased or	DERWENT;	
		efficient) adj3 (express or expression)	IBM TDB	
		same yeast)		
5	8	(alkaline adj phosphatase) same (express	USPAT;	2003/08/27
		or expression) and (((multiple or	US-PGPUB;	07:55
		several or different or many or	EPO; JPO;	
]		independent) adj3 (transformant or	DERWENT;	
]		transformation)) same (resistance or	IBM_TDB	
		selection)) and ((high or increased or	_	
		efficient) adj3 (express or expression)		
		same yeast))		
6	141	1 ,	USPAT;	2003/08/27
		yeast same alkaline adj phosphatase	US-PGPUB;	07:57
			EPO; JPO;	
			DERWENT;	
			IBM_TDB	
7	33	·	USPAT;	2003/08/27
		alkaline adj phosphatase	US-PGPUB;	07:56
[EPO; JPO;	
			DERWENT;	
8	1	/transformation on transformants\ ====10	IBM_TDB	2002/09/27
`	1	(transformation or transformants) adj10	USPAT;	2003/08/27
		alkaline adj phosphatase same yeast	US-PGPUB;	07:56
			EPO; JPO;	
			DERWENT; IBM TDB	
9	27	(transformation or transformants) adj10	USPAT;	2003/08/27
-	21	alkaline adj phosphatase and yeast	US-PGPUB;	07:56
[aliallic adj phosphacase and yease	EPO; JPO;	0,.50
			DERWENT;	
			IBM TDB	
10	24	((transformation or transformants) same	USPAT;	2003/08/27
	-	yeast same alkaline adj phosphatase) and	US-PGPUB;	07:58
		(express or expression) adj20 alkaline	EPO; JPO;	
		adj phosphatase	DERWENT;	
			IBM TDB	
11	8	eukaryotic adj3 alkaline adj phosphatase	USPAT;	2003/08/27
			US-PGPUB;	07:58
			EPO; JPO;	
			DERWENT;	
			IBM TDB	
12	173	(bovine or eukaryotic) adj5 alkaline adj	USPAT;	2003/08/27
		phosphatase	US-PGPUB;	07:59
			EPO; JPO;	
			DERWENT;	
			IBM_TDB	
	. — — —			

			·	
13	7	(bovine or eukaryotic) adj5 alkaline adj phosphatase same yeast	USPAT; US-PGPUB; EPO; JPO;	2003/08/27 08:00
			DERWENT; IBM_TDB	
14	3833	yeast adj10 expression adj system	USPAT; US-PGPUB; EPO; JPO;	2003/08/27 08:01
15	0	yeast adj10 expression adj system and	DERWENT; IBM_TDB USPAT;	2003/08/27
		(mulitple adj transformation)	US-PGPUB; EPO; JPO; DERWENT;	08:01
16	6	yeast adj10 expression adj system and (multiple adj transformation)	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:02
	5.0.5		IBM_TDB	2002/02/05
17	535	yeast adj10 expression adj system and multiple adj (transformation or copy or copies)	USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:04
18	77	yeast adj10 expression adj system and	IBM_TDB USPAT;	2003/08/27
	, ,	multiple adj (transformation or copy or copies)) and eukaryotic adj5 gene	US-PGPUB; EPO; JPO; DERWENT;	08:04
10	71		IBM_TDB	2002/00/07
19	71	eukaryotic same yeast adj10 expression same multiple adj (transformation or copy or copies)	USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:09
20	67	eukaryotic adj5 gene same yeast adj10 expression same multiple adj (transformation or copy or copies)	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:05
21	4	(eukaryotic same yeast adj10 expression same multiple adj (transformation or copy	IBM_TDB USPAT; US-PGPUB;	2003/08/27 08:08
		or copies)) not (eukaryotic adj5 gene same yeast adj10 expression same multiple adj (transformation or copy or copies))	EPO; JPO; DERWENT; IBM TDB	
22	1	producing adj10 eukaryotic same yeast same multiple adj (transformation or copy or copies)	USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:10
23	4103	yeast same multiple adj3transformations	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:10
24	24	yeast same multiple adj3 transformations	IBM_TDB USPAT; US-PGPUB; EPO; JPO;	2003/08/27 08:12
25	1771	method adj10 expression adj10 yeast	DERWENT; IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:13
			IBM_TDB	
26	0	method adj10 expression adj10 yeast same gene adj copy	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 08:14

<u> </u>			and the second of the Second S	
27	14124	method adj10 expression adj10 yeast and (increased or multiple or high) copy adj	USPAT; US-PGPUB;	2003/08/27 08:15
		number	EPO; JPO; DERWENT;	
28	80	method adj10 expression adj10 yeast and (increased or multiple or high) adj5 copy adj number	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:15
29	73	method adj5 expression adj10 yeast and (increased or multiple or high) adj5 copy adj number	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:16
30	4	(eukaryotic same yeast adj10 expression same multiple adj (transformation or copy or copies)) not (eukaryotic adj5 gene same yeast adj10 expression same multiple	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:16
31	70	adj (transformation or copy or copies)) method adj5 expression adj10 yeast and (increase or multiple or high) adj5 copy adj number	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 08:17
32	70	(method adj5 expression adj10 yeast and (increase or multiple or high) adj5 copy adj number) not (eukaryotic adj5 gene same yeast adj10 expression same multiple adj (transformation or copy or copies))	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 08:20
33	69	((method adj5 expression adj10 yeast and (increase or multiple or high) adj5 copy adj number) not (eukaryotic adj5 gene same yeast adj10 expression same multiple adj (transformation or copy or copies))) and (tranform or transformation or transforming)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/27 08:27
34	38	1	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/27 08:22
35	10	(((method adj5 expression adj10 yeast and (increase or multiple or high) adj5 copy adj number) not (eukaryotic adj5 gene same yeast adj10 expression same multiple adj (transformation or copy or copies))) and (tranform or transformation or transforming) same (marker)) and alkaline	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/27 08:22
36	8	adj phosphatase ((method adj5 expression adj10 yeast and (increase or multiple or high) adj5 copy adj number) not (eukaryotic adj5 gene same yeast adj10 expression same multiple adj (transformation or copy or copies))) and (transform or transformation or transforming) same (two or second) adj10 marker	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/27 08:28
37	0	(transform or transformation or transforming) same (two or second) adj10 marker same (high or ncrease or increased or efficient) adj5 expression adj5 yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 08:29
38	1	(transform or transformation or transforming) same (two or second) adj10 marker same (high or increase or increased or efficient) adj5 expression adj5 yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 08:29

39	13	(transform or transformation or	USPAT;	2003/08/27
		transforming) same (two or second) adj10	US-PGPUB;	08:34
		marker and (high or increase or increased	EPO; JPO;	
	:	or efficient) adj5 expression adj5 yeast	DERWENT;	
			IBM TDB	
40	0	marker same increasing adj5 (drug or	USPAT;	2003/08/27
		concentration) same yeast same copy adj	US-PGPUB;	08:35
		number	EPO; JPO;	
			DERWENT;	
			IBM_TDB	
41	15		USPAT;	2003/08/27
		concentration) and yeast same copy adj	US-PGPUB;	08:38
		number	EPO; JPO;	
	ŀ		DERWENT;	1
4.0	2511		IBM_TDB	2002/02/25
42	3511	, , , , , , , , , , , , , , , , , , , ,	USPAT;	2003/08/27
		concentration) or amplifiable) and yeast	US-PGPUB;	08:39
		same copy adj number and alkaline adj	EPO; JPO;	
		phosphatase or (two or second) adj marker	DERWENT;	
43	38	marker same /increasing adif /drug er	IBM_TDB	2002/09/27
43	30	marker same (increasing adj5 (drug or concentration) or amplifiable) and yeast	USPAT; US-PGPUB;	2003/08/27
		same copy adj number and (alkaline adj	EPO; JPO;	08.46
		phosphatase or (two or second) adj	DERWENT;	
		marker)	IBM TDB	
47	0	method adj10 alkaline adj phosphatase	USPAT;	2003/08/27
• ,		adj5 expression same eukaryotic	US-PGPUB;	08:47
		aujo empression sumo eunuryosio	EPO; JPO;	
			DERWENT;	
			IBM TDB	
48	3	method same alkaline adj phosphatase adj5	USPAT;	2003/08/27
		expression same eukaryotic	US-PGPUB;	08:49
			EPO; JPO;	
			DERWENT;]
			IBM_TDB	
49	1	(human or bovine) adj10 alkaline adj	USPAT;	2003/08/27
		phosphatase adj5 expression same	US-PGPUB;	08:49
		eukaryotic	EPO; JPO;	
			DERWENT;	
			IBM_TDB	
50	39	, , , , , , , , , , , , , , , , , , , ,	USPAT;	2003/08/27
		phosphatase adj5 expression	US-PGPUB;	08:50
			EPO; JPO;	
			DERWENT;	
			IBM_TDB	<u> </u>

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FILE 'HOME' ENTERED AT 10:06:28 ON 27 AUG 2003
=> file medline caplus
COST IN U.S. DOLLARS
                                                  SINCE FILE
                                                                  TOTAL
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                                                                SESSION
FULL ESTIMATED COST
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=> s alkaline (A) phosphatase
        100771 ALKALINE (A) PHOSPHATASE
=> s (bovine or hyman or eukaryotic) (5A) alkaline (A) phosphatase
           479 (BOVINE OR HYMAN OR EUKARYOTIC) (5A) ALKALINE (A) PHOSPHATASE
=> s 12 and express or produce (S) yeast
          3318 L2 AND EXPRESS OR PRODUCE (S) YEAST
=> s 12 and (express or produce (S) yeast)
             O L2 AND (EXPRESS OR PRODUCE (S) YEAST)
=> s (express or produce (S) yeast) and (amplifiable (A) marker)
             4 (EXPRESS OR PRODUCE (S) YEAST) AND (AMPLIFIABLE (A) MARKER)
=> d ibib abs 1-4
                       MEDLINE on STN
   ANSWER 1 OF 4
ACCESSION NUMBER:
                    2002133906
                                   MEDLINE
DOCUMENT NUMBER:
                    21823394 PubMed ID: 11834126
                    High-level expression of human thyroid-stimulating hormone
TITLE:
                    in Chinese hamster ovary cells by co-transfection of
                    dicistronic expression vectors followed by a dual-marker
                    amplification strategy.
AUTHOR:
                    Peroni Cibele N; Soares Carlos R J; Gimbo Elizabeth;
                    Morganti Ligia; Ribela Maria Teresa C P; Bartolini Paolo
CORPORATE SOURCE:
                    Biotechnology Department, National Nuclear Energy
                    Commission (IPEN-CNEN), Travessa R-400, Cidade
                    Universitaria, 05508-900, Sao Paulo, SP, Brazil.
SOURCE:
                    BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (2002 Feb) 35 (Pt
                    1) 19-26.
                    Journal code: 8609465. ISSN: 0885-4513.
PUB. COUNTRY:
                    England: United Kingdom
DOCUMENT TYPE:
                    Journal; Article; (JOURNAL ARTICLE)
                    English
LANGUAGE:
                    Priority Journals
FILE SEGMENT:
                    200205
ENTRY MONTH:
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Last Updated on STN: 20020502

Entered Medline: 20020501

AB The utilization of dicistronic mRNA expression vectors, containing

Entered STN: 20020301

ENTRY DATE:

The utilization of dicistronic mRNA expression vectors, containing the gene of interest upstream of an amplifiable marker gene, has shown success in rapidly, efficiently and reproducibly obtaining stable cell lines that express high levels of the protein of interest. For this reason, human thyroid-stimulating hormone (hTSH), a heterodimeric glycoprotein composed of non-covalently linked alpha- and beta-subunits, was expressed in Chinese hamster ovary (CHO) cells using a system based on dicistronic expression vectors. These contained the genes of interest and the amplifiable gene markers dihydrofolate reductase (DHFR) and adenosine deaminase (ADA), separated by an internal ribosome entry site isolated from the encephalomyocarditis virus. After the cells (CHO-DHFR-) had been co-transfected with the expression vectors and submitted to gene amplification in culture medium containing stepwise increments of methotrexate, it was possible to isolate clones that presented a secretion level of up to 7.2+/-1.3 microg/10(6) cells per day, the highest ever reported for the expression of this glycoprotein hormone. A second treatment, involving the utilization of deoxycoformycin, directed to amplify the ADA marker gene, provided a clone with an additional 2-3-fold increase in hTSH secretion, reaching a secretion level of 17.8+/-7.6 microg/10(6) cells per day. Cell culture and hTSH production in a hollow-fibre bioreactor were set up in order to carry out a preliminary physico-chemical, immunological and biological characterization of this hormone in comparison with pituitary-extracted hTSH (from the National Institute of Diabetes and Digestive and Kidney Diseases) and the only recombinant hTSH now available (Thyrogen). The availability of recombinant hTSH is very important in the diagnosis and

therapy of thyroid carcinoma, via stimulation of radioiodine uptake.

L5 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: DOCUMENT NUMBER:

2002:354032 CAPLUS

136:351379

TITLE:

Expression constructs comprising multiple units of

promoter linked to exon and unpaired splice sequence

and uses for improved gene expression

INVENTOR (S):

Harrington, John J.

414,369, abandoned.

20020509

PATENT ASSIGNEE(S):

SOURCE:

A1

U.S. Pat. Appl. Publ., 43 pp., Cont. of U.S. Ser. No.

CODEN: USXXCO

DOCUMENT TYPE:

Patent

LANGUAGE: FAMILY ACC. NUM. COUNT: 1

English

PATENT INFORMATION:

PATENT NO. KIND DATE

APPLICATION NO. DATE ______

US 2002055172 PRIORITY APPLN. INFO.:

US 2000-729416 20001205 US 1999-414369 B1 19991007

The present invention is directed to improved methods for gene expression using genetic vectors with at least two units, each unit comprising multiple promoters operably linked to an exon and unpaired splice sequence. Multiple promoter/exon units, which produce multiple RNA transcripts, are used in nucleic acid constructs to provide increased expression of a desired nucleic acid sequence. The sequence is introduced into a vector by conventional cloning or is expressed from an endogenous sequence in the genome that is activated by the vector contg. the multiple promoters. The vectors can be used to express cDNA clones, genes encoded by genomic DNA or fragments, activate endogenous genes in situ, and modify gene or protein of interest.

ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER:

2002:201542 CAPLUS

DOCUMENT NUMBER:

136:354224

TITLE:

High-level expression of human thyroid-stimulating

hormone in Chinese hamster ovary cells by co-transfection of dicistronic expression vectors

AUTHOR (S):

followed by a dual-marker amplification strategy Peroni, Cibele N.; Soares, Carlos R. J.; Gimbo,

Elizabeth; Morganti, Ligia; Ribela, Maria Teresa C.

P.; Bartolini, Paolo

CORPORATE SOURCE:

Biotechnology Department, National Nuclear Energy Commission (IPEN-CNEN), Sao Paulo, 05508-900, Brazil

SOURCE:

Biotechnology and Applied Biochemistry (2002), 35(1), 19-26

CODEN: BABIEC; ISSN: 0885-4513

PUBLISHER:

Portland Press Ltd.

DOCUMENT TYPE: LANGUAGE:

Journal English

The utilization of dicistronic mRNA expression vectors, contg. the gene of interest upstream of an amplifiable marker gene, has shown success in rapidly, efficiently and reproducibly obtaining stable cell lines that express high levels of the protein of interest. For this reason, human TSH (hTSH), a heterodimeric glycoprotein composed of non-covalently linked .alpha.- and .beta.-subunits, was expressed in Chinese hamster ovary (CHO) cells using a system based on dicistronic expression vectors. These contained the genes of interest and the amplifiable gene markers dihydrofolate reductase (DHFR) and adenosine deaminase (ADA), sepd. by an internal ribosome entry site isolated from the encephalomyocarditis virus. After the cells (CHO-DHFR-) had been co-transfected with the expression vectors and submitted to gene amplification in culture medium contg. stepwise increments of methotrexate, it was possible to isolate clones that presented a secretion level of up to 7.2.+-.1.3 .mu.g/106 cells per day, the highest ever reported for the expression of this glycoprotein hormone. A second treatment, involving the utilization of deoxycoformycin, directed to amplify the ADA marker gene, provided a clone with an addnl. 2-3-fold increase in hTSH secretion, reaching a secretion level of 17.8.+-.7.6 .mu.g/106 cells per day. Cell culture and hTSH prodn. in a hollow-fiber bioreactor were set up in order to carry out a preliminary physico-chem., immunol. and biol. characterization of this hormone in comparison with pituitary-extd. hTSH (from the National Institute of Diabetes and Digestive and Kidney Diseases) and the only recombinant hTSH now available (Thyrogen). The availability of recombinant hTSH is very important in the

diagnosis and therapy of thyroid carcinoma, via stimulation of

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L5 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1987:418844 CAPLUS

DOCUMENT NUMBER: 107:18844

TITLE: Use of the Escherichia coli gene for asparagine

synthetase as a selective marker in a shuttle vector capable of dominant transfection and amplification in

animal cells

Cartier, Mireille; Chang, Mildred W. M.; Stanners, AUTHOR (S):

Clifford P.

CORPORATE SOURCE: Cancer Cent., McGill Univ., Montreal, QC, H3G 1Y6.

SOURCE: Molecular and Cellular Biology (1987), 7(5), 1623-8

CODEN: MCEBD4; ISSN: 0270-7306

DOCUMENT TYPE: Journal

LANGUAGE: English

A new dominant amplifiable selective system for use in bacterium-animal cell shuttle vectors was developed by the insertion of a 2-kilobase genomic fragment contg. the cloned E. coli gene for asparagine synthetase (AS) into the pBR322-simian virus 40 recombinant vector pSV2 so as to place the translational initiator codon for the bacterial AS about 1,000 base pairs downstream from the simian virus 40 early promoter. This new construct, pSV2-AS, retains bacterial sequences for transcriptional and translational initiation and so can express AS in bacteria. The construct can also complement AS- mutants of mammalian cells, giving AS+ transfectants capable of growth in medium lacking asparagine, with relatively high efficiency (about 300 colonies per .mu.g of DNA per 106 cells exposed). The vector can be amplified up to 100-fold in such AS+ transfectants by selection in asparagine-free medium contg. increasing concns. of the AS inhibitor .beta.-aspartyl hydroxamate. AS+ transfectants were found to be much more resistant to a second AS inhibitor, Albizziin, than were normal AS+ animal cell lines. This difference, which may indicate a strong resistance of the bacterial AS enzyme to Albizziin, was exploited to develop an effective selection for bacterial AS transfectants of a no. of wild-type AS+ cell lines of rat, Chinese hamster, mouse, and human origin. LR-73 cells, a Chinese hamster AS+ cell line, were transfected with pSV2-AS with an efficiency of about 1,000 colonies per 0.5 .mu.g of DNA per 106 cells. The integrated construct in these cells was amplified by incubation of the transfectants in increasing concns. of .beta.-aspartyl hydroxamate. Advantages and disadvantages of this new dominant, selectable, and amplifiable marker over markers commonly used in shuttle vectors are discussed.

```
=> s (express or produce (S) yeast) and (multiple (5A) marker)
            70 (EXPRESS OR PRODUCE (S) YEAST) AND (MULTIPLE (5A) MARKER)
L6
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- => s (express or produce (S) yeast) and (multiple (5A) marker) (S) increase (S) (copy or gene) L7 O (EXPRESS OR PRODUCE (S) YEAST) AND (MULTIPLE (5A) MARKER) (S) INCREASE (S) (COPY OR GENE)
- => s (express or produce (S) yeast) and (multiple (5A) marker) and increase (S) (copy or gene) 0 (EXPRESS OR PRODUCE (S) YEAST) AND (MULTIPLE (5A) MARKER) AND INCREASE (S) (COPY OR GENE)